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FINAL REPORT

**MYCOPLASMA BOVIS AND BOVINE VIRUS DIARRHEA
VIRUS IN FEEDLOT CATTLE DYING WITH HEART LESIONS**

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***"A Study of the Prevalence of *Hemophilus somnus*, *Mycoplasma bovis*
and Bovine Viral Diarrhea Virus in Feedlot Cattle Dying with Heart
Lesions in Myocarditis"***

Abstract

The objectives of this study were to determine the occurrence of bacterial pathogens as causative agents in feeder cattle dying from myocarditis and to determine if an association existed between these death losses and infection with bovine virus diarrhea virus (BVDV). Tissues from the heart and lungs of 92 calves dying with myocarditis in Alberta feedlots were immunohistochemically stained for antigens of *Hemophilus somnus*, *Mycoplasma bovis*, *Mannheimia hemolytica* and bovine viral diarrhea virus. Tissues from 44 calves dying from noninfectious causes and 35 calves dying with pneumonia were tested as controls. *Hemophilus somnus* was found in cardiac lesions in the majority of myocarditis cases (70/92). *Mycoplasma bovis* was demonstrated in the hearts of 4/92 affected calves. No bacterial pathogens were found in heart tissues from control groups of calves. BVDV infection was demonstrated in 4/92 myocarditis cases compared to 13/35 pneumonic calves and 0/45 noninfectious death losses. The results demonstrate that *Hemophilus somnus* is the principle pathogen involved in myocarditis in feedlot calves and that the concurrent presence of BVDV is increased in these calves compared to calves dying of noninfectious causes.



Executive Summary

Feeder cattle have large individual value so it is critical to minimize their death losses to maintain profitability of this industry. About one third of all death losses in feeder cattle are due to lesions in the heart muscle. The lesions are obviously bacterial and *Hemophilus somnus* is the agent that has been classically isolated from the tissues.

At the WCVI we have specialized in the development of immunohistochemical (IHC) methods for demonstration of infectious agents. Immunohistochemical methods are staining techniques which enable identification of infectious agents without the need to isolate the organism. This has lead to the ability to demonstrate agents which are difficult to isolate by traditional culture methods. One such organism that is notoriously difficult to isolate is *Mycoplasma bovis*. The ability to identify this agent through IHC stains has enabled our group to demonstrate that many chronic pneumonia and polyarthritis death losses in feeder cattle are associated with this agent, rather than with *Hemophilus somnus* as was often previously assumed. Further, we have been able to show a strong association between the occurrence of chronic death losses in feeder cattle and the presence of the immunosuppressive virus, bovine viral diarrhea virus.

These findings have prompted us to question the presumption that all the feeder cattle losses associated with myocarditis were due to *Hemophilus somnus*. We speculated that *Mycoplasma bovis* might also have a causative role and that BVDV might be also acting as a precipitating cause as in other feeder cattle death losses.

The findings of the study were that *Hemophilus somnus* was the primary bacterial pathogen involved in the myocarditis deaths. *Mycoplasma bovis* was present only in a minority of cases. This confirms the previous presumptions and does not explain why these losses occur in spite of vaccination for *Hemophilus somnus* and vigorous anti-microbial therapy. The occurrence of BVDV virus was higher in the cattle dying with myocarditis than in those dying from noninfectious causes supporting the hypothesis that the effects of the BVDV virus predisposes to increased risk of death from myocarditis. This association, however, was not as strong as with other types of death losses previously linked to BVDV.

Some of the most interesting data in this study emerged from the testing of the control group of cattle. We tested 2 groups of controls for the myocarditis losses, a group of cattle dying from noninfectious causes and a group of cattle dying early in the feeding period with pneumonia. In the pneumonic group of cattle a variety of bacterial agents were found in the lungs, as expected, and in addition a very high proportion were shown

to have concurrent BVDV infections. This is the first association of the BVDV virus with pneumonic losses occurring early in the feeding period. Our previous data had focused on the role the virus was playing in chronic pneumonia/polyarthrits losses late in the feeding period. This is important as it again refocuses our attention on the very important role that BVDV is playing in contributing to death losses in the feedlot.

Disappointingly, vaccination did not protect cattle against death losses from either myocarditis or pneumonia. This result suggests that better strategies are needed if vaccination is going to have a role in prevention of these infectious death losses in the feedlots.

Technical Report

Introduction

Sudden death in feedlot cattle associated with multi-focal myocardial infarcts, necrosis and fibrosis was first been described as a syndrome associated with septicemic infection with *Hemophilus somnus* in the late 1980s (Janzen 1987, Harris and Janzen 1989, Guichon et al, 1988). In spite of vaccination of incoming calves for *Hemophilus somnus* and vigorous antibiotic treatment of febrile animals, heart lesions including myocarditis, pericarditis, pleuritis continues to be an important cause of calf mortality in fall placed calves in western Canadian feedlots (van Donkersgoed et al., 1990, 1994, Pollack et al., in press).

In some studies all losses associated with laryngitis, myocarditis, pericarditis, pleuritis, polyarthritis and septicemia have been attributed to infection with *Hemophilus somnus* (van Donkersgoed et al, 1990, 1994, Booker et. al., 1999), however this has been presumptive as the agent was not cultured or identified by immunohistochemistry. Recently, there has been emerging evidence that *Mycoplasma bovis*, possibly secondary to acute BVDV infection is involved in a high proportion of losses in feeder calves (Pollack et. al., in press, Haines et al, in press, Shahriar et al, in press). In particular, the presumption that chronic nonresponsive polyarthritis is due to *Hemophilus somnus* infection has been challenged by findings that the majority of these cases are immunohistochemically positive for the antigens of *Mycoplasma bovis* and negative for those of *Hemophilus somnus*. A high proportion of these cases have also been shown to have concurrent BVDV infection.

The purpose of the study described in this report was to determine, using immunohistochemical stains, the prevalence of *Mycoplasma bovis* and *Hemophilus somnus* in the lesions of feeder calves dying with myocardial lesions and to determine the numbers of these calves concurrently infected with BVDV.

Material and Methods

The calves eligible for inclusion in the study were all those that died in the fall of 1999/winter of 2000 in Alberta feedlots serviced by the veterinarians participating in the study. Five veterinary practices participated in the study and samples were collected from 10 feedlots.

Post mortem examination of 182 dead cattle was conducted by a participating veterinarian and a gross diagnosis used to categorize the case as either: myocarditis (n=92), pneumonia (n=35), noninfectious (bloat or atypical interstitial pneumonia), or "other" ailment (n=11). The losses categorized as "other" were excluded from further analysis. The additional information recorded for each case included the following: the numbers of days on feed and vaccination for any or all of the following agents: bovine viral diarrhea virus, bovine respiratory syncytial virus, bovine herpes type I virus, *Pasteurella hemolytica* (*Mannheimia*) *hemolytica*, and *Hemophilus somnus*.

The following samples were harvested and placed in 10% neutral buffered formalin: 2 x 2 cm samples of haired skin of the neck, a 2 x 2 cm sample of lung including any consolidated tissues and/or obvious lesions, a 1-2 cm wide sample from the wall of the right ventricle, from the left intra-ventricular septum and 2 samples from the wall of the left ventricle (the latter including the papillary muscles). The participating veterinarians were also instructed to sample any area of the heart with an obvious abscess or inflammation of the surface of the myocardium.

The tissue samples were embedded in paraffin wax and sectioned for immunohistochemical staining using staining methods previously described (Haines and Chelack, 1990). Duplicate sections from each block were tested for each antigen of the following 4 pathogens: *Hemophilus somnus*, *Pasteurella* (*Mannheimia*) *hemolytica*, *Mycoplasma bovis* and bovine viral diarrhea virus. The antisera used were the following: 1. *M. bovis*: polyclonal rabbit antiserum at dilutions of 1/1000 and 1/2000 (a gift from the Diagnostic Bacteriology Laboratory, Western College of Veterinary Medicine, Saskatoon, SK), 2. *M. heme*: monoclonal antibodies P12/D6/D5 diluted 1/2000 and 1/4000 (a gift from The Veterinary Infectious Diseases Organization, Saskatoon, SK), 3. *H. somnus*: rabbit polyclonal antiserum diluted 1/1000 and 1/2000 (a gift from The Veterinary Infectious Diseases Organization, Saskatoon, SK), 4. BVDV: monoclonal antibody 15C5 at dilutions of 1/800 and 1/1600 (a gift from Dr E. Dubovi, Cornell University, Ithaca, NY). Positive control sections from isolation-confirmed cases were tested concurrently with test tissues. Negative controls were tissue sections from each

block tested with substitution of an irrelevant polyclonal antiserum or monoclonal antibody. Tissues were scored as either positive or negative.

Results:

Table 1 shows the numbers of days in the feedlot of the death losses in each group. The losses associated with myocarditis and pneumonia most often occurred early in the feeding period (mean days on feed 43 and 39, respectively) while the losses associated with noninfectious causes occurred most often later in the period (mean number of days on feed was 100).

The relationship between vaccination and the group of death loss is shown in Table 2. Vaccination for *Hemophilus somnus* was more common in calves dying with either myocarditis (Odds Ratio = 10.93) or pneumonia (Odds Ratio = 4.271) compared to noninfectious causes. Vaccination for BVDV was also more common among calves dying with myocarditis (Odds Ratio = 4.348) and pneumonia (Odds Ratio = 2.22) than animals dying of noninfectious causes. Vaccination for *Mannheimia hemolytica* was similar among all groups of calves.

Tables 3 & 4 shows the numbers of cases in each group of death losses in which the 4 pathogens were detected in either the heart or the lung. *Haemophilus somnus* was demonstrated in the lesions in the heart in the majority of cases of myocarditis (70/92) (Figure 1). In 4 cases of myocarditis *M. bovis* was detected within the cardiac lesions (Figure 2), in these cases there was concurrent *H. somnus* infection. *Mannheimia hemolytica* was not detected in the heart. There was no detection of bacteria in the heart tissues of calves dying from either noninfectious causes or from pneumonia.

In pneumonic losses, *Mannheimia hemolytica* was detected in about half the cases (Table 4). The other bacteria (*Hemophilus somnus* and *Mycoplasma bovis*) were also detected in the lungs in substantial numbers of cases. In 5 cases there was concurrent detection of *M. bovis* and *M. heme* (data not shown).

Bovine viral diarrhea virus was found in 4/92 cases of myocarditis (Figure 3), this is a significantly increased rate compared with the 0/44 animals dying of noninfectious causes (Odds Ratio = 20.52). However BVDV was detected in pneumonia cases (13/35) more often than in myocarditis cases (Odds ratio = 13.00). The most compelling association with BVDV was demonstrated in the cases dying with pneumonia compared to those cases with noninfectious deaths (Odds Ratio = 261).

Discussion

Hemophilus somnus was shown to be associated with the lesions in the heart tissues of the majority of myocarditis death losses in feeder cattle. This is in agreement with most previous reports and presumptions. While *Mycoplasma bovis* is increasingly recognized as having a role in the feeder calf death losses associated with chronic pneumonia and polyarthritis, this bacterium was shown to be present in only a minority of the myocarditis cases (and with concurrent *Hemophilus somnus* infection) suggesting that *Hemophilus somnus* is the primary causal bacterium.

For more than a decade there has been evidence emerging that increased risk of disease in feedlot cattle may be associated with BVDV infections. Serological studies have demonstrated that sero-conversion to BVDV during the feeding period is related to increased risk of disease (Martin et al, 1990) and higher incoming titers to BVDV protect calves from subsequent mortality (Booker, 1999, O'Conner, 2001). Several recent retrospective and prospective studies from the Western College of Veterinary Medicine have demonstrated an association between BVDV and *Mycoplasma bovis* in the chronic pneumonia and polyarthritis-induced losses in feeder (Pollack et al in press, Haines et al in press, Shahriar et al, in press). Emerging from the present study is additional evidence for an association of BVDV with feeder cattle losses. BVDV was found more frequently in cattle dying with both myocarditis and pneumonia. While the association between myocarditis losses and the presence of BVDV was not as strong as that found with pneumonia losses and with chronic death losses previously reported, there is nonetheless evidence that this virus may be increasingly the numbers of losses due to *H. somnus* induced myocarditis. In addition, in the current study the pneumonic losses were most often early in the feeding period suggesting that BVDV may be predisposing to these deaths as well as to those occurring in association with chronic unresponsive disease later in the feeding period.

The relationship between vaccination and group of death loss demonstrated that, contrary to expectation, vaccinated animals were more likely to die from infectious agents than from noninfectious causes. This suggests that more effective vaccination strategies are necessary to protect cattle against *Hemophilus somnus* myocarditis as well as pneumonia induced death losses.

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Table 1 The numbers of days in the feedlot prior to death in groups of feeder cattle

Group	Numbers of Days in Feedlot		
	Mean	SD	Median
Mycocarditis (n=92)	43	21	41
Pneumonia (n=35)	39	44	18
Noninfectious (44)	100	48	100

Table 2 Vaccination status in groups of feeder cattle death losses

Group	Vaccination for infectious agents		
	<i>H. somnus</i>	<i>M. hemolytica</i>	BVDV
Mycocarditis (n=92)	76/84	40/80	70/84
Pneumonia (n=35)	26/33	14/31	23/32
Noninfectious (44)	20/43	16/42	23/43

Table 3 Immunohistochemical demonstration of *Hemophilus somnus*, *Mycoplasma bovis*, *Manneheimia hemolytica*, and Bovine Viral Diarrhea Virus from Heart Tissues of Feeder Cattle

Immunohistochemical detection of:				
Group	<i>H. somnus</i>	<i>M. hemolytica</i>	<i>M. bovis</i>	BVDV
Mycocarditis (n=92)	70/92	0/92	4/92	4/92
Pneumonia (n=35)	0/35	0/35	0/35	13/35
Noninfectious (44)	0/44	0/44	0/44	0/44

Table 4 Immunohistochemical demonstration of *Hemophilus somnus*, *Mycoplasma bovis*, *Manneheimia hemolytica*, and Bovine Viral Diarrhea Virus from Lung Tissues of Feeder Cattle

Immunohistochemical detection of:				
Group	<i>H. somnus</i>	<i>M. hemolytica</i>	<i>M. bovis</i>	BVDV
Mycocarditis (n=92)	5/92	1/92	6/92	4/92
Pneumonia (n=35)	9/35	17/35	9/35	13/35
Noninfectious (44)	0/44	0/44	0/44	0/44

Project Developed Materials and Photos

Figure 1. Photomicrograph of the immunohistochemical stain of *Hemophilus somnus* in a myocarditis case. Avidin biotin complex immuno-peroxidase stain developed with diaminobenzadine with a hematoxylin counterstain.



Figure 2. Photomicrograph of the immunohistochemical stain of *Mycoplasma bovis* in a myocarditis case. Avidin biotin complex immuno-peroxidase stain developed with diaminobenzadine with a hematoxylin counterstain.



Figure 3. Photomicrograph of the immunohistochemical stain of Bovine Viral Diarrhea Virus in a myocarditis case. Avidin biotin complex immuno-peroxidase stain developed with diaminobenzadine with a hematoxylin counterstain.



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Expense Statement